CHAPTER I

INTRODUCTION AND LITERATURE REVIEWS

Aloe vera

Botany of aloe vera

The aloe plant belongs to the lily family, family Liliaceae and subfamily Aloineae, which comprises more than 600 species (Winters and Yang, 1996). The common names are called Mediterranean aloe, True aloe, Star cactus, Waan haang jarake, Waan faimai (Northern) and Haang takhe (Central) (Norman and Bunyapraphatsara, 1992). The nomenclature of aloe vera had been very confused and the plant had been known under a variety of names (Reynolds, 1966). Later the scientific name was concluded as Aloe vera (Linn.) Burm .f. (Grindlay and Reynolds,1986). Only a few species of more than 600 species were currently used by the pharmaceutical or cosmetic industries. These three main species are Aloe forrox (Mill.), Cape Aloe ; Aloe perri (Baker), Zanzibar Aloe, Socotrine Aloe ; and Aloe barbadensis (Mill), Aloe vera (Linne), Aloe vera (Spoerke and Ekin, 1980).

Aloe vera is a short-stemmed succulent herb. The succulent leaves are crowded on the top of their stems, spreading, grayish green and glaucous ; spotted when young, 20-50cm long, 3-5cm wide at the base, tapering gradually to the point tip, 1-2.5cm thick; having edeges spiny, and bitter latex inside. Flowers borne on the upper part of a slender stalk, 50-100cm high. Forms of the species vary in sizes of leaves and colors of flowers (Grindlay and Reynolds, 1986).

The epidermis of the leaves has a thick cuticle, and beneath is a zone of parenchyma which obtains pericyclic cells. The latex or yellow juice contains

within the pericyclic cells. The central bulk of the leaf contains the colourless mucilaginous pulp, made up of large thin-walled mucilaginous cell containing the aloe gel itself (Klein and Penneys, 1988) as shown in figure 1.

Chemical constituents.

Several studies of chemical constituent analysis in the latex portion have found anthraquinone glycosides derivatives such as aloin, barbaloin, isobarbaloin, anthranol, aloe-emodin, chrysophanic acid, 1, 8 - dihydroxyanthraquinone (Henry, 1979 ; Hirata and Suga, 1977 ; Robson et al., 1982 ; Spoerke and Ekin, 1980).

The fresh gel had been found to consist of 99.5% water and 0.5 % solid component (Gjerstad, 1971; Mckeown, 1983). Analysis of the solid components revealed that the largest number of active substances (97%) were in the mixed polysaccharides (Davis et al., 1981 ; Yagi, 1982) such as glucose, mannose, galactose, xylose, and arabinose (Waller et al., 1978). Besides, the solid components had been found to comprise glycoprotein, aloctin A, aloctin B (Suzuki et al., 1979); amino acids, such as aspartic acid, glutamic acid, serine, threonine, asparagine, glutamine, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine (Waller et al., 1978), uric acid, salicylic acid, creatinine, alkaline phosphate, cholesterol, triglyceride, lactate, calcium, magnesium lactate, zinc, sodium, potassium, chloride (Bouchey and Gjerstand, 1969; Hirata and Suga, 1977; Robson et al., 1982); enzymes such as amylase, bradykininase, catalase, cellulase, oxidase (Fugita, 1976; Leung, 1977; Rowe and Parks, 1941), vitamin c (Davis et al., 1990).

It was believed that a strong synergistic relationship existed between polysaccharides and other active substances in aloe such as amino acids and vitamins (Davis et al., 1990 ; Henry, 1979 ; Leung, 1977, 1978 ; Waller, 1978), Certain amino acids and vitamins showed strong anti-inflammatory activity (Hanley et al., 1982), suggesting that these substances might have a triggering effects on enzymes and polysaccharides activity needed for antiinflammation (Coats, 1979). The healing properties of aloe vera as well as the antiinflamtory effects of aloe vera had polysaccharide base as active ingredient, and also needed synergistic effect of their active substances (Henry, 1979; Leung, 1977, 1978; Mckeown, 1983; Waller, 1978).

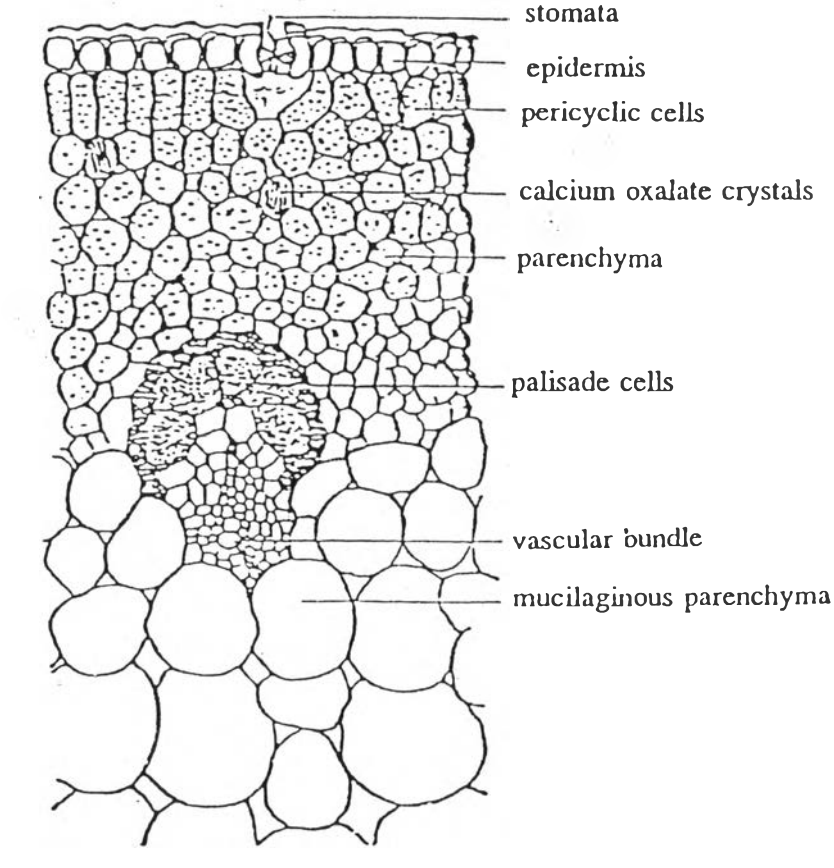

Figure 1.1 A transverse section near the margin of the aloe leaf (Klein et al .,1988)

Pharmacological activities

Since Biblical times use of aloe vera as a remedy had repeatedly come up in folklore, along with testimonials related to the "healing" properties of the mucilage when applied to burned or inflamed skin. The beneficial effects of aloe were so miraculous as to seen more like myth than fact (Cole and Chen, 1943). For the last several decades, the scientists had begun seriously probing aloe chemisty for "non-folkore".

Today, aloe vera has been recognized as a good medicine which has properties including analgesia, anticancer, antiviral, mutagen, antiulcer, cough suppressant,hyperglycemia, antifungal, anthelmintic, antiarthritis, antiparasite, antifertility, cathartic, cosmetics, insecticide, fat production and decongestion (Norman and Bunyapraphatsara, 1992). Regarding the therapeutic effects of aloe vera on inflammation, it was investigated in many studies as the followings.

Physiological activities

Prostaglandins and thromboxanes might play role in the long-term inflammatory response in tissue injuries. They had a number of different physiological effects including vasoconstriction, promotion of fever and pain, and they also had an influence on the immune system. Prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) were vasodilator and platelet aggregation inhibitor. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), thromboxane A_2(TXA_2) and thromboxane B_2(TXB_2) were vasoconstrictors and platelet aggregators (Heggers and Robson, 1983, 1985).

Robson et al. (1980) investigated the capability of synthetic drugs to inhibit prostaglandin and thromboxane productions in the secondary degree burn model. Burn wounds were examined by means of immunoperoxidase technique for the presence of prostaglandins and thromboxanes. The burned tissue showed high levels of PGE_2 and TXA_2. Using three thromboxane inhibitor drugs i.,e., imidazole, methimazole, and dipyridamole,it was shown that PGE_2, $PGF_{2\alpha}$ and PGI_2 had similar levels in untreated-burned tissue, but TXA_2 was essentially absent, suggesting that thromboxane might be responsible for the progressive dermal ischemia after burning and that decreasing its production could increase dermal perfusion.

Progressive dermal ischemia resulting in progressive cell injury and death was classical phenomenon of the burn wound for 24 to 48 hours following the thermal insult (Jackson, 1953). Leukocyte sticking to vessel walls, agglutination of red blood cells , and liberation of vasoactive and necrotizing substances occured following burn injury. The damaged tissue underwent vascular sludging, thrombosis, progressive dermal ischemia, and death. Decreasing the progressive dermal ischemia could theoretically limit the amount of skin loss to those cells irreversibly coagulated at the time of burning (Robson et al., 1980).

Steriods blocked the formation of arachidonic acid by inhibiting phospholipase. Aspirin and indomethacin acted on the next step of prostaglandin synthesis by blocking cyclooxygenase, an enzyme that acted to convert arachidonic acid into prostaglandins. Thus, using phospholipase inhibitor drugs and cyclooxygenase inhibitor drugs inhibited PGI_2, PGE_2, $PGF_{2\alpha}$, PGD_2 and TXA_2 (Robson et al., 1980). It was suggested that suppression thromboxane could avoid the side effects and could improve dermal perfusion (DelBeccaro et al., 1980). By blocking TXA_2 which limited

the side effects, platelet adherence was reduced, white blood cells and red blood cells were prevented from sticking to vessel walls, and vasoconstriction was inhibited (Moncada, 1978; Needleman et al., 1977).

Many studies have revealed that aloe vera has both antithromboxane and antiprostaglandin activities. Several vehicle components which had the lyophilized aloe gel and fresh gel in their components were tested for the inhibition of arachidonic acid oxidation in vitro by using oxygen monitor assay. Several vehicle components and aloe vera were found to be complex lipid which could inhibit the oxidation of arachidonic acid, reflecting inhibition of lipoxygenase and/or prostaglandin synthesis activity or, possibly, sequestration of arachidonic acid. Similar effects could occur in vivo as well (Penneys, 1982). In the full thickness canine burn model, burn wounds were examined by immunohistochemical determination of prostaglandins. In untreated burn wounds showed marked presence of TXB_2 and some PGE_2 and $PGF_{2\alpha}$. In aloe, treated burn wounds showed TXB_2 diminishing in intensity and finally disappearing, while PGE_2 and $PGF_{2\alpha}$ appeared to increase and maintain an apparent equilibrium. Aloe vera could reduce vasoconstriction and preserve the dermal vasculature (Cera et al., 1980). This result was confirmed by Heggers and Robson (1982) but instable TXA_2 was determined instead of stable TXB_2.

Besides, Robson et al. (1982) investigated the effects of aloe vera on thermal burn wound in a standard guinea pig experiment. The depth of dermal ischemia was measured by perfusion with Indian ink. The result was that aloe vera had similar effects to methylprednisolone and methimazole, giving improved perfusion of capillaries and reduction in TXB_2 and $PGF_{2\alpha}$ compared to the control animals which showed complete dermal ischemia by 24 hrs. Heggers and Robson (1983) suggested that aloe vera gel products

contained anthraquinone and related compounds such as barbaloin and aloe-emodin in sufficient quantities to act as competitive inhibition as called false substrate inhibitor blocking prostanoid synthesis, since they had a similar chemical structure to prostaglandin substrates. Moreover, Hegger and Robson (1985) postulated that the stereochemical configurations of the aloe vera and anthraquinone-like agent were similar and the presence of the appropriate carboxyl, hydroxyl or oxygen molecule combined to create a false substrate inhibitor. On the other hand, Capasso et al. (1983) found that aloin and 1,8-dioxyanthraquinone stimulated prostaglandin production in isolated rat colon, this effect was thought to contribute to the laxative effect of these compounds. This result was controversial to the investigation of Heggers and Robson (1983). Consequently, it was suggested that large concentration of anthraquinone-type compounds increased prostaglandin levels but trace amounts, as in aloe vera gel, inhibited prostaglandin synthesis. Alternatively, skin and colon tissue could have different physiological responses (Grindlay and Reynolds, 1986).

Progressive dermal ischemia had been observed in other models as well (Heggers et al., 1993) such as frostbite injury (McCauley et al., 1983 ; Miller et al., 1995), electrical injury (Robson et al., 1984), and intra-arterial drug abuse (Zachary et al., 1987). These models showed that the mediators of progressive tissue damage were thromboxanes. Experimentally aloe vera was compared to a variety of specific or nonspecific thromboxane blocking agents. In each model, aloe vera and therapeutic agents were found to increase tissue survival or preserve the tissue necrosis, by actively inhibited the localized production of thromboxanes. Yet aloe vera was found less toxic than therapeutic agents. Thus, aloe vera not only acted as a TXA_2 inhibitor but also maintained a homeostasis within the vascular endothelium as well as the surrounding tissue (Heggers et al. 1993). Moreover, Afzal et al. (1991)

found the presence of cyclooxygenase enzyme in aloe vera extracts. By having established arachidonic materials, potential precursors for the prostanoids synthesis were mixed with preincubated supernatant aloe. The presence of cyclooxygenase was demonstrated by the conversion of [^{14}C] arachidonic acid into different prostanoids including 5.3% keto-PGF_1b_α, 10.36% $PGF_{2\alpha}$, 19.23% TXA_2, 52.66% PGE_2, and 11.80% PGD_2.

Several pharmacological studies have been performed in an attempt to identify active substance for antiinflammatory action of aloe vera. In vivo study of Fujita and Shosuke in 1976 found that lyophilized powder aloe contained bradykininase. This result was comfirmed by Rubel (1983) that the bradykininase activity of aloe vera could hydrolyze bradykinin and angiotensin I to convert into angiotensin II, resulting in suppressing vasodilation and pain. The carboxy peptidase was reported to be enzyme in aloe vera gel that could hydrolyze bradykinin and angiotensin I in vitro (Fujita et al., 1979). Bradykinin was both a vasodilator and potent pain-producing agent at the site of acute inflammation. The carboxypeptidase from aloe could inhibit bradykinin in vivo, yet decreasing pain at the site of acute inflammation (Klein and Penneys,1988). The antibradykinin active material in aloe vera which was tested on isolated guinea pig ileum in vitro, was estimated to be a glycoprotein (Yagi et al., 1982). It was suggested that aloe glycoprotein had the presence of carboxypeptidase *N*-and *P*-like enzymes with proteolytic activity. These results might provide a pharmaceutical basis for the antiinflammatory action of aloe vera (Yagi et al., 1986).

Hirata and Suga (1977) found magnesium lactate in aloe vera. In vivo study demonstrated that magnesium lactate inhibited the conversion of histidine to histamine in mast cells by inhibiting histidine decarboxylase (Lehninger, 1981). This result was comfirmed by Rubel (1983) that inhibition

of histidine decarboxylase by magnesium lactate in aloe might result in decreasing vasodilation. Nakagomi et al. (1984) tested the inhibitory effects of aloein, barbaloin and seven aloe extracts on isolated rat mast cell degranulation by measuring histamine release and porcine platelet aggregation. All of these agents did not cause platelet aggregation nor inhibit the aggregation induced by ADP or collagen. They also had no activities to release histamine from mast cells. However, inhibitory activities on histamine release from mast cell induced by compound 48/48 were found in all the samples tested. Barbaloin had much stronger inhibitory activity compared to aloein for their effects on histamine release from mast cell. Thus, biological activity of barbaloin and aloe extracts was found to have inhibitory effects on histamine release from mast cells suggesting their usefulness as antiinflammatory substances in in vivo system.

Aloe vera was shown to be effective antiinflammatory and antiedemic agent. The carragenan was first used to induce edema formation by Winter et al. in 1962. Carragenan was colloidal extract obtained from red marine algae. It was composed of a mixture of salts of an acid sulfate of a galactose-containing polysaccharide. The hind paws of Wistar albino rats were induced edema using carragenan. The polysaccharide, mannan or 1,4-linked β-D mannopyranose, from the nondialysate of aloe vera inhibited edema. The percentages of swelling at 1,2,3,4, and 5 hrs, in treated with aloe mannan group were 22.3% , 19.1%, 20.3%, 37.1% and 48.9%, respectively, while those of untreated edema group were 24.8%, 43.5%, 63.1%, 62.9% and 72.4%, respectively (Yagi et al., 1984). Aloe vera provided the substance acetylsalicylic acid which had antiinflammatory and antiedemic activity by blocking prostaglandin synthesis (Davis et al., 1986). Saito et al. (1982) discovered that the lectin aloctin A in aloe vera inhibited biosynthesis of PGE_2 from arachidonic acid which caused inflammation, resulting in reduced

edema and adjuvant arthritis in rats. Davis et al.(1986) showed certain amino acids, vitamins and RNA in 50% ethanol supernatant aloe to have antiinflammatory activity. Danof (1987) believed that glycoprotein in the 50% ethanol supernatant aloe had antiinflammatory activity. Besides, aloe had antiinflammatory components such as amino acids (Forst and Davis, 1979), vitamins (Davis et al., 1990), aspirin-like agent (Coats, 1979), and mannose (Willenberg et al., 1989) which could normalize the acute vascular response.

Aloe vera preparations were evaluated for topical antiinflammatory activity by the croton oil-induced edema assay. The decolorized aloe with removal of anthraquinone inhibited edema. As shown by unit of edema volume, edema in decolorized aloe-treated group was significantly decreased when compared with untreated-edema group and diabetic edema group (Davis et al., 1988). The decolorized aloe was more effective than the colorized aloe with anthraquinone by 47% inhibition of inflammation (Davis et al., 1989). Davis et al. (1991) investigated the preparations of aloe vera which had antiinflammatory activity. They prepared aloe vera extract with 50% ethanol, resultant supernatant and precipitate were tested for antiinflammatory activity using the croton oil - induced ear-swelling assay. When applied topically, the percentage of decreased inflammation was 29.2% for supernatant fraction and 12.1% for precipitate fraction. Thus, the antiinflammatory activity (inhibitory system) resided in the supernatant of a 50% ethanol extract. However, this experiment found that the precipitate fraction decreased the wound diameter by an average reduction of 47.1% (stimulatory system). Furthermore, Davis et al. (1994) showed that the aloe vera sterols, lupeol, campesterol, and β - sitosterol, had significantly antiinflammatory effects. Of the three sterols, lupeol caused the greatest reduction in inflammation by 37.0%. β - sitosterol reduced inflammation by 37.1% and campesterol reduced inflammation by 24.2%. Lupeol alone reduced inflammation in

dose-dependent manner. Little or no wound healing activity was found in the supernatant (Davis et al., 1991, 1994).

Recently, Vazquez et al. (1996) studied the effects of aqueous, chloroform and ethanol extracts of aloe vera gel. The chemical group, anthraglycosides, reductor sugars, cardiotonic glycosides, mucilaginous an pectins, of aqueous extract of aloe vera inhibited in vitro conversion of arachidonic acid to PGE_2, suggesting antiinflammatory activity on the arachidonic acid pathway via cyclooxygenase. Likewise, the chemical group sterols type Δ^5 and anthraquinones of chloroform extract and the chemical group of aqueous extract decreased the edema induced in the hind paw. Hutter et al. (1996) found a new antiinflammatory agent as cinnamoyl-*C*-glucosylchromone. Using croton oil-induced ear inflammation model, at a dose of 200 μg/ear of cinnamoyl-*C*-glucosylchromone exhibited topical antiinflammatory activity equivalent to 200 μg/ear of hydrocortisone (Hutter et al., 1996).

Polymorphonuclear leukocyte functions related to the process of wound healing were phagocytosis, chemotaxis and bacteriocidal activity (Rubinstein, 1983). Moreover, the bacteriocidal activity was mediated in part by the production of reactive oxygen species and in part by oxygen-independent mechanisms (Robbins et al., 1994).

A variety of studies have shown wound healing properties of aloe vera. Coats (1979) and Engel et al. (1987) found amino acid, ascorbic acid, zinc, lignins, and saponins presented in aloe vera which increased the synthesis of collagen and counterbalanced collagen breakdown with subsequent increase in wound tensile strength. The fresh aloe vera leaves had lectin-like compounds which enhanced the growth of normal human cell in tissue culture

(Winter et al., 1981). Using full thickness burned wound model maintained temperature at 250°C for 6 seconds by hot plate, the procedure was designed to compare the effect of aloe vera extract, silvadine, salicylic acid cream and untreatment. The average time to complete healing in the untreated group was 50 days and the only significant difference was found in the aloe vera - treated group. which healed on an average time of 30 days. In addition, wound bacterial counts were effectively decreased by silvadine and by aloe vera extract (Rodriguez-Bigas et al., 1988).

Davis et al. (1988) compared the percentage of wound reduction among varied doses of decolorized aloe vera group, control group and diabetic group on the seventh day and fourteenth day. The diameters of the inflicted wound were measured with vernier caliper. The results in each decolorized aloe vera groups showed significantly increased rates of healing in dose-related manner on both seventh day and fourteenth day compared with control group and diabetic group. Aloe vera at doses of 100 and 300 mg/kg daily for 4 days effectively decreased hydrocortisone's antiwound healing properties (Davis et al., 1994). It was found that aloe vera stimulated fibroblasts to increase in number in a dose-response fashion. These findings would tend to indicate strength of wounds by producing collagen of the fibroblasts (Danof, 1987; Heggers et al., 1993; Winter et al., 1981). The ethanol precipitate which had mannose as major carbohydrate (Gowda, 1979) decreased the wound diameter by an average of reduction 47.1% (Davis, 1991). The growth factors including gibberellin, auxins and mannose-6-phosphate, which were identified in aloe vera , were effective in promoting wound healing (Davis et al., 1994 ; Morgan et al., 1987).

Visuthikosol et al. (1995) studied in twenty-seven patients with partial thickness burn wound which were treated with aloe vera gel compared with

vaseline gauze on the first day, seventh day, fourteenth day, twenty-first day or until complete epithelialization. Wound healing appeared to have macroscopic hyperemia, edema, and bleb formation in both aloe gel-treated area and non-treated area during the first week. Within the fourteenth day, most of the aloe vera gel-treated area showed complex healing and only some cases of deep partial thickness burn might show epithelial islands of burn wound. The average healing time of the aloe vera gel-treated area was 11.89 days and the vaseline gauze-treated area was 18.18 days. In histologic study, on the seventh day after treatment the ulcerated surface of both areas was covered with necrotic tissue, debris and red blood cells. In addition, the vaseline gauze-treated area was also covered by acute inflammatory exudate intermixed with necrotic tissue. Epithelialization was fully developed on the fourteenth day of aloe vera gel-treated area, while the vaseline gauze-treated area had partially developed in specimens. On the twenty-first day, the complete development of epidermis and dermis was evident after aloe vera gel treatment. The granulation tissue and inflammatory exudate in dermis were placed by newly developed fibro-vascular and collagen tissue. These findings were not seen in the vaseline gauze-treated area.

Recently, Yagi et al. (1997) found that glycoprotein fraction promote cell growth, while the neutral polysaccharide fraction did not show any growth stimulation.

Concerning bactericidal activity of aloe vera, Lorenzetti et al. (1964) found that aloe vera powder significantly inhibited growth on plates inoculated with Staphylococcus aureus, Streptococcus pyrogens, Corynebacterium xerose and Salmonella paratyphi. Robson et al. (1982) found that 60% concentration of aloe vera extract inhibited Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, Citrobacter species,

Enterobacter cloacae, S. pyogens, and Streptococcus agalactiae, 80% for Escherichia coli, and 90% for Streptococcus faecalis and Candida albicans.

Furthermore, the studies had been performed in an attempt to clarify the mechanisms of bactericidal activity of aloe vera. Shida et al. (1985) studied the effects of the active components, i.e, glycoprotein and polysaccharide fraction, of aloe vera extract on phagocytosis in blood samples of patients with bronchial asthma. Only glycoprotein fraction clearly enhanced the phagocytic activity in a dose-dependent fashion. The aqueous, chloroform and ethanol extract of aloe vera not only showed antiinflammatory activity but also decreased the number of neutrophils migrating into the peritoneal cavity. The aqueous extract decreased migration by 28.6% and the chloroform extract by 42.9% (Vazquez et al., 1996). Aloe vera extract had been identified two distinct components. One component inhibited the production of free radical by polymorphonuclear leukocyte (PMN) called inhibitory system, whereas a second component stimulated antibody production called stimulatory system. ('t Hart et al., 1988, 1989, 1990).

Recently, Sabeh et al. (1993) found glutathione peroxidase present in aloe vera gel and also found superoxide dismutase from gel and the rind of the aloe vera (Sabeh et al., 1996).

From the literature reviews mentioned above, it was obvious that aloe vera has been found to contain various kinds of active components. It could be used to treat inflammation and wound healing. However, the antiinflammatory mechanisms underlying the therapeutic effects of aloe vera is largely unknown. Therfore, it is the hypothesis of the present study that the active components in gel may inhibit vasodilation caused by released vasoactive substances including histamine, bradykinin, in the early phase of inflammation at the site of injury. Besides, these components may enhance

vasodilation by inhibition vasoactive mediators such as TXA_2 in the late phase of inflammation.

Therefore, the objectives of this study are:

1). To study the effects of aloe vera on wound healing area.

2). To study the effects of aloe vera on arteriolar diameter changes.

3). To study the effects of aloe vera on postcapillary venular permeability and leukocyte adhesion.